

Testosterone Perturbs Epidermal Permeability Barrier Homeostasis

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Although there are no known gender-related differences in permeability barrier function in adults, estrogens accelerate whereas testosterone retards barrier development in fetal skin, and male fetuses demonstrate slower barrier development than female littermates. Moreover, prenatal administration of the androgen receptor antagonist, flutamide, equalizes developmental rates in male and female fetuses. Therefore, we evaluated the effects of changes in testosterone on barrier homeostasis in adult murine and human skin. Hypogonadal mice (whether by castration or by treatment with systemic flutamide) displayed significantly faster barrier recovery at 3, 6, and 12 h than did controls, and testosterone replacement slowed barrier recovery in castrated mice. Moreover, testosterone directly effects the skin, as topical flutamide also accelerated barrier recovery in normal male mice. These findings appear to be of physiologic significance, since prepubertal male mice (age 5 wk) displayed accelerated barrier recovery in comparison

with adult postpubertal (11 wk) males. These studies also appear to be relevant for humans, as a hypopituitary human subject demonstrated repeated changes in barrier recovery in parallel with peaks and nadirs in serum testosterone levels during intermittent testosterone replacement. Mechanistic studies showed that differences in epidermal lipid synthesis do not account for the testosterone-induced functional alterations. Instead, epidermal lamellar body (LB) formation and secretion both decrease, resulting in decreased extracellular lamellar bilayers in testosterone-replete animals. These studies demonstrate that fluctuations in testosterone modulate barrier function, and that testosterone repletion can have negative consequences for permeability barrier homeostasis. **Key words:** androgens/epidermal lipid synthesis/epidermal ultrastructure/lamellar bodies/permeability barrier/testosterone/trans-epidermal water loss. *J Invest Dermatol* 116:443–451, 2001

The cutaneous permeability barrier resides in the outermost layer of the epidermis, the stratum corneum (SC). The SC is a highly resilient tissue organized into a unique two-compartment system of nucleated corneocytes embedded in a lipid-enriched, intercellular matrix, which is enriched in ceramides, cholesterol, and free fatty acids (Elias and Menon, 1991). This hydrophobic mixture is organized into lamellar bilayer stacks, which mediate both transcutaneous water loss and the percutaneous absorption of xenobiotics. Acute and chronic perturbations in the permeability barrier stimulate a coordinated, lipid synthetic and secretory

response in the underlying epidermis that leads to rapid restoration of barrier homeostasis (Elias and Feingold, 1992; Elias, 1996). Yet, little is known about the regulation of epidermal barrier homeostasis by circulating hormones or other biologic response modifiers.

In addition to their well-known effects on the pilosebaceous apparatus (Ebling, 1957), androgens influence epidermal growth and differentiation. Keratinocytes both express androgen receptors and possess the enzymatic apparatus to convert testosterone to its active metabolite, dihydrotestosterone (Wilson and Lasnitzki, 1971; Takayasu *et al*, 1980; Luu-The *et al*, 1994; Eicheler *et al*, 1995; Thiboutot *et al*, 1995). Moreover, prior studies have shown that androgens stimulate epidermal hyperplasia (Zackheim, 1968), and modulate epidermal differentiation (Tammi, 1982), apparently due to an increase in the number of cells in the granular layer (Tammi and Santti, 1989). Furthermore, sex hormones exert important influences on the late stages of permeability barrier development *in utero* (Williams *et al*, 1998). Whereas exogenous estrogens accelerate barrier ontogenesis both *in utero* and in growth-factor-free explant cultures, androgens delay barrier ontogenesis (Hanley *et al*, 1996). In addition, male fetal mice display a delay in barrier

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Abbreviations: EDTA, ethylene diamine-tetraacetic acid; LB, lamellar body; SC, stratum corneum; TEWL, transepidermal water loss; TNS, total nonsaponifiable lipids.

development *versus* female littermates, an effect that is reversible when pregnant mothers are treated with the testosterone receptor antagonist, flutamide (Hanley *et al*, 1996): although decreased survival rates in male premature infants have been ascribed to increased respiratory distress rates (Khouri *et al*, 1985), a negative influence of androgens on skin development could explain ongoing outcome differences in premature human male *versus* female infants, even when treated with surfactant replacement therapy (Allen *et al*, 1993; La Pine *et al*, 1995).

These observations in fetal skin prompted us to examine the potential influence of modulations in circulating testosterone on permeability barrier function in adult skin. Using the well-characterized murine model, we demonstrated differences in barrier homeostasis in testosterone-replete *versus* -deficient mice, and in one hypogonadal human subject.

MATERIALS AND METHODS

Materials Castrated, sham-operated, and normal hairless male mice (Sk:h1) were purchased from Charles River Laboratories (Philadelphia, PA). Adult mice, age 11–14 wk, were used for all experiments. Serum testosterone levels were >1500 pg per ml in control animals and less than 20 pg per ml in castrated animals (IDEXX Veterinary Services, West Sacramento, CA). Prepubertal male mice were utilized between 4 and 5 wk of age. Testosterone propionate and flutamide was purchased from Sigma (St. Louis, MO). Subcutaneous testosterone and flutamide were solubilized in commercial peanut oil. Topical flutamide was solubilized in propylene glycol:ethanol (7:3 vols) at the final concentrations, volumes, areas, and routes of administration described in the results and figure legends for each experiment. Peanut oil and propylene glycol:ethanol were used as vehicles, respectively.

One 58-y-old, hypogonadal male subject (post-transphenoidal hypophysectomy and radiation therapy in 1984) was studied at various points before and after testosterone replacement (testosterone cypionate 200 mg IM at 3-wk intervals). Serum testosterone levels were measured in this subject and in the castrated animals by standard radioimmunoassay.

Measurements of barrier recovery and SC integrity Transepidermal water loss (TEWL) was measured with an electrolytic water analyzer (Meeco, Warrington, PA). Barrier function was disrupted by sequential cellophane tape stripping until TEWL levels reached 3–8 mg per cm^2 per h (Khouri *et al*, 1985; Allen *et al*, 1993). TEWL was then measured immediately after acute barrier disruption and at various time intervals thereafter, usually at 1, 3, 6, and 12 h after barrier disruption. SC integrity was defined as the mean number of tape strippings required to abrogate the barrier to ≥ 3 mg per cm^2 per h (Ghadially *et al*, 1995).

Lipid synthesis studies For nonpolar lipid synthesis studies, animals ($n=8$ each) first were killed 1 h after barrier disruption. One centimeter square skin samples then were obtained, and incubated immediately for 2 h at 37°C in a 2-ml solution of 10 mM EDTA in Dulbecco's PBS, calcium and magnesium free, containing 40 μCi [^{14}C] acetate, as described previously (Monger *et al*, 1988). After stopping the reaction by immersion in iced PBS, the epidermis was separated from the dermis, and the quantities of labeled fatty acids and cholesterol were determined in the epidermis after saponification, extraction, and thin layer chromatography (Grubauer *et al*, 1987; Mao-Qiang *et al*, 1993). Results are expressed as nanomoles incorporated per hour per gram epidermis.

For glucosylceramide synthesis, animals ($n=6$ each) were sacrificed without prior barrier disruption, and 9.6 cm^2 samples were excised. The subcutaneous fat was removed by scraping with a scalpel, and the samples were incubated immediately for 2 h at 37°C in a 3-ml solution of 0.07 mM Ca^{++} keratinocyte culture medium, containing 30 μCi [^3H] galactose (American Radiolabeled Chemicals, St. Louis, MO) (Komori *et al*, 1999). The samples were removed from the incubation medium to stop reactions, and immersed in calcium/magnesium-free PBS at 60°C for 60 s to separate the epidermis from the dermis. Total lipids were extracted from the epidermis using the Bligh-Dyer method, as described previously (Mao-Qiang *et al*, 1993), and glucosylceramide fractions were separated further using thin layer chromatography (Holleran *et al*, 1991). The results are expressed as nanomoles of galactose incorporated per hour per gram epidermis.

Light microscopy, ultrastructural, and quantitative morphometric studies Skin biopsy samples were taken immediately before and 3 h after tape stripping of castrated mice, treated with either subcutaneous

testosterone or vehicle (see above; $n=3$ from each group), and processed for light and electron microscopy. Samples were minced to <0.5 mm^3 , fixed in modified Karnovsky's fixative overnight, and postfixed in either 0.5% ruthenium tetroxide or 2% aqueous osmium tetroxide, both containing 1.5% potassium ferrocyanide, as described previously (Hou *et al*, 1991). After fixation, all samples were dehydrated in graded ethanol solutions, and embedded in an Epon-epoxy mixture. One-half micron sections, stained with toluidine blue, were used for light microscopic studies. Epidermal thickness and the number of nucleated cell layers in the epidermis was measured over ≥ 20 sites each from androgen-replete *versus* vehicle-treated, castrated mice in coded micrographs by an unbiased observer. Ultrathin sections were examined, with or without further contrasting with lead citrate, in an electron microscope (Zeiss 10 A, Carl Zeiss, Thornwood, NY) operated at 60 kV.

For quantitative ultrastructural studies, 10–21 pictures of the outer stratum granulosum (SG) and the SG-SC interface at a constant original magnification of 15,000 \times were selected at random from each sample group. Lamellar body density per micrograph was calculated as the number of lamellar bodies per unit area. Assignment of organelles as lamellar bodies required the presence of a trilaminar limiting membrane, characteristic ellipsoidal shape, and a 0.4–0.6 μm long axis. The nucleus was excluded from calculations of cytosolic volume. The cross-sectional area of the cytosol was measured by integrating area measurements on the scanned pictures with NIH image software Ver.1.62 (<http://rsb.info.nih.gov/nih-image>). The extent of lamellar body secretion was quantified as the cross-sectional area of the intercellular domain at the SG-SC junction divided by the measured length in micrometers of the measured area. The cross-sectional area was determined by two unrelated methods: (a) weighing paper overlays of the region (Rassner *et al*, 1999); and (b) integrating area measurement on the scanned pictures with NIH image software.

Statistical analysis For statistical analyses, we utilized two-tailed and paired Student's *t* tests with software from SPSS (Chicago, IL). Data are expressed as mean \pm SEM with $p \leq 0.05$ considered significant.

RESULTS

Barrier recovery and integrity change with testosterone depletion/repletion To assess the impact of androgens on permeability barrier homeostasis, we first compared the kinetics of barrier recovery in castrated *versus* sham-operated hairless mice.

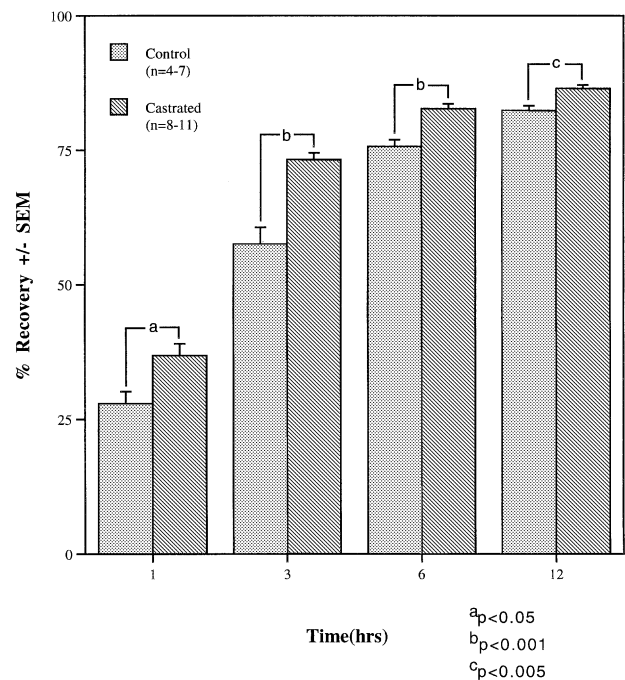


Figure 1. Barrier recovery improves after surgical castration of male mice. Barrier recovery was compared 8 wk after castration or sham-operation of hairless male mice. Data shown represent mean \pm SEM; ^a $p < 0.05$; ^b $p < 0.001$; ^c $p < 0.005$.

Table I. SC integrity in castrated, testosterone-replete *versus* control mice

| Animals | Tape strippings \pm SEM ^a |
|-----------------------------------|--|
| Non-castrated (n = 7) | 3.29 \pm 0.18 ^b |
| Castrated + vehicle (n = 11) | 4.27 \pm 0.14 ^b |
| Castrated + testosterone (n = 11) | 2.27 \pm 0.14 ^b |

^aNumber of strippings required to abrogate barrier to TEWL \geq 3 mg per cm² per h.

^bDifferences between all groups are significant at $p < 0.001$.

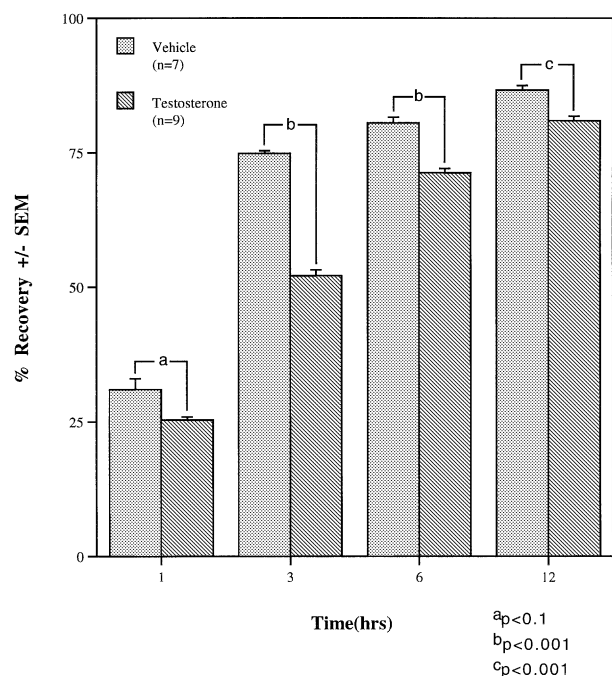


Figure 2. Barrier recovery worsens in castrated male mice after systemic testosterone replacement. Castrated mice were injected subcutaneously with either testosterone propionate (5 mg per kg) diluted in peanut oil or peanut oil alone daily for 7 d. Barrier recovery was measured on the eighth day. Data represent mean \pm SEM; ^a $p < 0.1$; ^b $p < 0.001$.

Barrier recovery was assessed 1, 3, 6, and 12 h after acute disruption by sequential tape stripping. As seen in **Fig 1**, the kinetics of barrier recovery was accelerated significantly at all time points in castrated mice, with the greatest differences apparent at 1 and 3 h (**Fig 1**; $p < 0.001$ and < 0.05 , respectively). Moreover, castrated animals displayed significantly greater SC integrity than control animals (**Table I**). These results show that surgically induced hypogonadism leads to accelerated barrier recovery and enhanced SC integrity in male mice.

To ascertain further whether accelerated barrier recovery after castration is due to androgen depletion, we next assessed the effects of testosterone- *versus* vehicle-repletion in castrated mice. Either testosterone (5 mg per kg) or vehicle was administered subcutaneously once daily for 7 d followed by measurement of barrier recovery after tape stripping. Whereas vehicle treatment did not influence barrier recovery rates, testosterone administration slowed barrier recovery at all time points (**Fig 2**), with the greatest difference at 3 h ($p < 0.001$). Likewise, testosterone treatment caused a significant decrease in SC integrity (**Table I**). These results show that testosterone replenishment reverses the acceleration in barrier recovery and improvement in SC integrity that occurs in castrated mice.

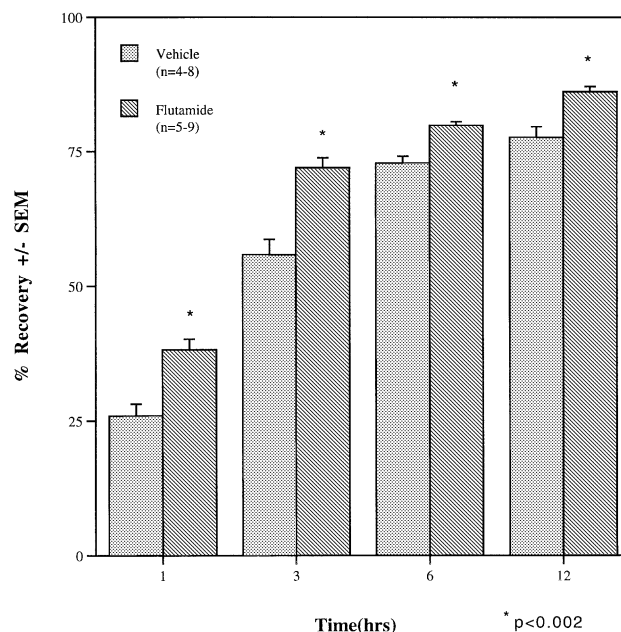


Figure 3. Barrier recovery in male mice improves after systemic flutamide treatment. Male mice were injected subcutaneously with flutamide (50 mg per kg) in peanut oil or the peanut oil vehicle alone for 7 d. Barrier recovery was measured on the eighth day. Data shown represent mean \pm SEM; * $p < 0.002$.

To determine whether the castration-induced acceleration of barrier recovery is specific for surgical castration, or a more general attribute of testosterone deficiency, we next assessed the effects of medical castration on barrier recovery in normal (noncastrated) male mice. For these studies, we administered either the androgen-receptor antagonist, flutamide (50 mg per kg), or vehicle alone subcutaneously once daily for 7 d, followed by measurement of barrier recovery on the eighth day. As seen in **Fig 3**, barrier recovery was accelerated at all time points in the flutamide-treated animals. In fact, the absolute recovery rates at each time point were virtually identical for medical castration to those in surgically castrated animals. These results show that testosterone depletion has positive effects on permeability barrier homeostasis, independent of the method of production of hypogonadism.

Testosterone influences are directed at the skin We next assessed whether the cutaneous effects of testosterone are due to systemic alterations, or whether they are directed at the skin itself. We were unable to utilize testosterone topically for these studies, because of bulk lipid effects of this steroid hormone on barrier function, which interfered with an assessment of hormone effects. Hence, we measured barrier recovery after seven, once-daily applications of flutamide (30 μ g per ml) to a 2.5-cm² area on one flank of normal, noncastrated mice. The contralateral flank was treated daily with an equal volume of the propylene glycol:ethanol vehicle alone. Topical flutamide treatment accelerated barrier recovery, whereas the vehicle applied to the contralateral flank did not alter barrier recovery rates *versus* vehicle applied to nonflutamide-treated mice (**Fig 4**). Because flutamide-treatment only affected the treated flank (the contralateral flank did not change significantly), these results show that the effects of testosterone are due to local, rather than distant effects of the hormone.

Adverse effects of testosterone are demonstrable with puberty and in testosterone-treated humans We next asked whether the large differences in endogenous androgen levels that occur in pre- *versus* postpubertal animals are associated with changes in barrier homeostasis. Prepubertal

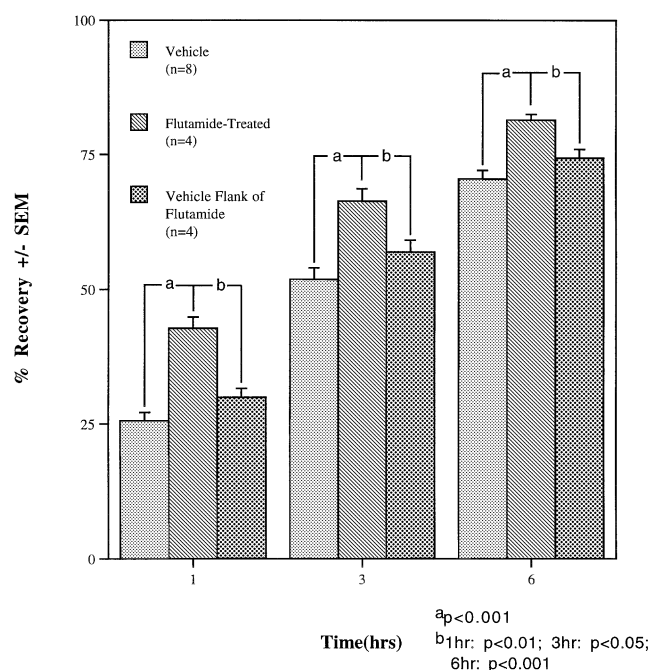


Figure 4. Barrier recovery in male mice improves after topical flutamide treatment. Male hairless mice were treated with topical flutamide in propylene glycol:ethanol vehicle (30 μ g per ml) on one (2.5 cm²) flank and vehicle alone on the opposite flank daily for 7 d, or vehicle alone applied to one flank of otherwise untreated mice daily for 7 d. Barrier recovery was measured on the eighth day. Data shown represent mean \pm SEM; ^a $p < 0.001$; ^b1 h: $p < 0.01$; 3 h: $p < 0.05$; 6 h: $p < 0.001$.

mice display a 60%–70% reduction in serum testosterone levels *versus* postpubertal animals (Overpeck *et al*, 1978). As seen in **Fig 5**, prepubertal mice (5 wk) display more rapid barrier recovery rates than do young adult male mice (11 wk) at both 3 and 6 h after acute disruption ($p < 0.02$). Recovery rates in the 11-wk-old mice again were comparable with those in the somewhat older (12–14 wk), noncastrated adults described above (cf. **Figs 1–4**). These results show that developmental changes in male gonadal status are paralleled by changes in barrier function.

To ascertain whether the adverse effects of androgens are of potential relevance for humans, we next assessed barrier recovery kinetics in one hypogonadal subject, who was receiving intermittent testosterone replacement (testosterone cypionate 200 mg IM q. 3 wk). Measurements of barrier function coincided with points of high (2 or 3 d after injections) *versus* low (immediately prior to next injection) serum testosterone levels. As seen in **Fig 6**, barrier recovery rates were highest when serum testosterone levels approached their nadir, while conversely, the kinetics of recovery slowed in conjunction with high testosterone blood levels. Although all observations were from a single subject, they achieved statistical significance ($p = 0.011$; by paired *t* test). These results suggest that permeability barrier homeostasis in humans also changes with alterations in serum testosterone.

Testosterone induces alterations in epidermal thickness and a decline in lamellar body production/secretion Finally, we assessed several potential mechanisms that could account for the effects of androgens on barrier homeostasis. Light microscopy revealed a slight, but significant decrease both in epidermal thickness and in the number of nucleated cell layers in testosterone- *versus* vehicle-treated, castrated animals (**Table II**). The synthesis of both nonsaponifiable (primarily cholesterol) and saponifiable (fatty

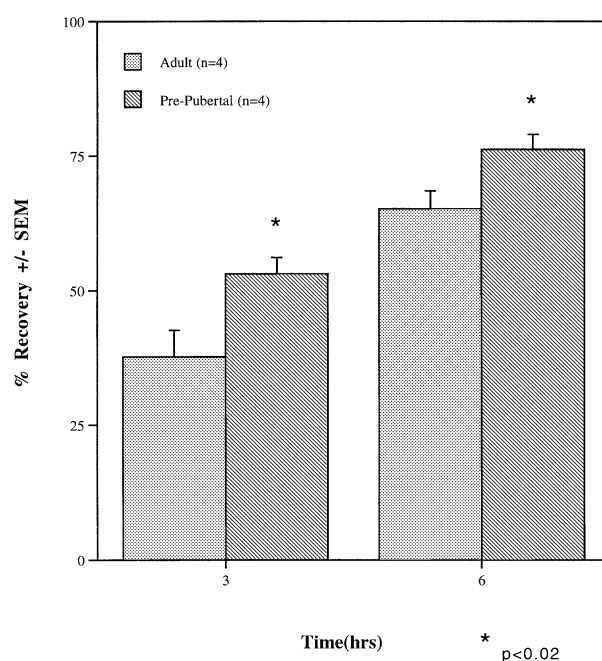


Figure 5. Barrier recovery is faster in pre-pubertal than in young adult male mice. The kinetics of barrier recovery was measured in 4-wk-old and 11-wk-old normal male hairless mice. Data shown represent mean \pm SEM; * $p < 0.02$.

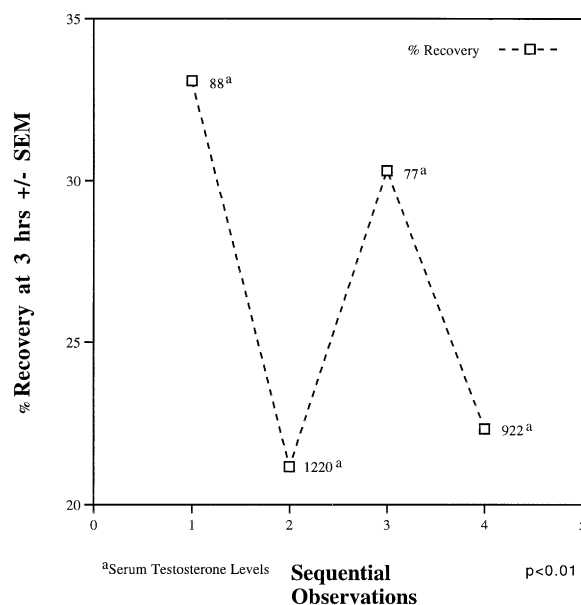


Figure 6. Changes in barrier recovery in relation to testosterone therapy in a hypogonadal human male. Barrier recovery during the “high” testosterone period was measured 3 d after intramuscular injection of testosterone cypionate (200 mg). (Vertical arrows indicate time points of testosterone administration.) Barrier recovery during the “low” testosterone period was measured 3 wk after the last prior testosterone injection. Serum testosterone was obtained at the same time as barrier recovery measurements. Statistical significance was calculated with the Student-*t* test where each observation was considered as an individual event; $p < 0.01$

acids) epidermal lipids, however, was comparable in castrated and noncastrated animals (**Table III**). Thus, testosterone repletion is accompanied by a decline in epidermal thickness, but modulations in cholesterol and fatty acid synthesis do not

account for the decline in barrier homeostasis in testosterone-replete animals.

We next assessed three ultrastructural markers of the lamellar body secretory system, i.e., lamellar body density/lamellar body secretion, and extracellular lamellar membrane structure, as potential mechanisms that could explain the testosterone-induced abnormality in barrier homeostasis. Both visual assessment and quantitative stereologic (morphometric) measures of randomly obtained, coded micrographs (observer-blinded) were employed. Whereas we did not observe consistent differences in the ultrastructure of untreated, normal *versus* vehicle-treated, castrated mice in the basal state, we did find differences in the ultrastructure of the epidermis in testosterone-

replete *versus* vehicle-treated castrated and control mice, despite the fact that TEWL rates were comparable under basal conditions (**Fig 7**). The density of lamellar bodies (LB) in the cytosol of cells in SG layers in testosterone-replete animals appeared to be reduced in comparison to vehicle-treated, castrated mice (**Fig 7C, D**). These observations were supported by quantitative (stereologic) measurements, which showed a significant (25%–30%) reduction in the volume fraction of LB in the cytosol of outermost SG cells (**Fig 8**; $p < 0.05$). In addition, the amount of secreted contents at the SG–SC interface also appeared to be reduced in testosterone-replete *versus* control animals (**Fig 7C vs 7D**). Consistent with reduced secretion, we found entombed LB within the cytosol of

Table II. Epidermal thickness in testosterone-replete *versus* vehicle-treated, castrated mice

| | Vehicle-treated | Sign. | Testosterone-replete |
|-----------------------|-----------------|------------|--------------------------|
| Thickness (μm) | 24.75 ± 1.2 | p < 0.005 | 17.86 ± 0.86 (n = 18–25) |
| Nucleated cell layers | 2.28 ± 0.11 | p < 0.0001 | 1.67 ± 0.11 (n = 27) |

n = number of measurements on multiple, randomly obtained, and assessed skin sections from two mice in each group (see *Materials and Methods*).

Table III. Lipid synthesis in castrated *versus* sham-operated mice

| Animals | Synthesis (nmol per gm epidermal weight per h) ^a | | |
|---------------|---|-------------|--------------------|
| | Cholesterol | Fatty acids | TNS |
| Castrated | 1.37 ± 0.23 | 6.72 ± 0.60 | 1.59 ± 0.29(n = 8) |
| Sham-operated | 1.21 ± 0.25 | 6.95 ± 0.85 | 1.40 ± 0.30(n = 8) |

^aDifferences are not significant; TNS = total nonsaponifiable lipids.

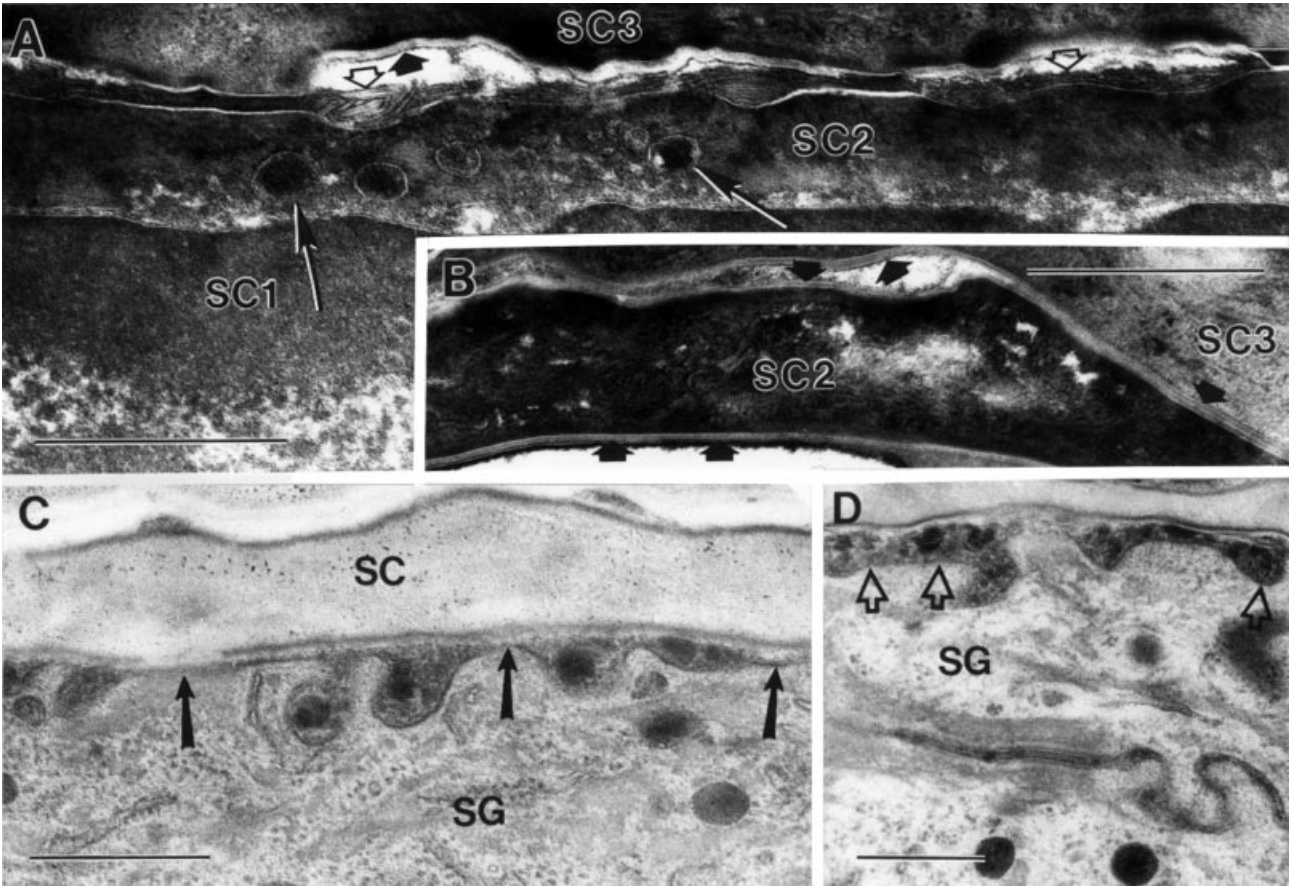


Figure 7. Lamellar body number and secretion appear to be impaired in testosterone-supplemented, castrated mice. (A, C) Castrated plus testosterone-treated; (B, D) castrated plus vehicle-treated skin. In testosterone-treated animals note: (1) decreased numbers of lamellar bodies in the cytosol of the outermost granular cell (SG); (2) decreased secreted lamellar contents at the SG–SC interface (C, arrows); (3) entombed lamellar bodies in the corneocyte cytosol (A, long arrows); and (4) delayed extracellular processing (A, open arrows depict unprocessed lamellar material; solid arrows indicate foci where processing into mature lamellae has occurred. Note that processing is still not completed between the second and third SC cell layer [A, SC2 and SC3]). In vehicle-treated animals, more lamellar bodies appear to be present in the cytosol (D); more secreted material appears to be deposited at the SG–SC interface (D, open arrows); and processing to mature lamellar membranes is complete by the SC1–SC2 interface (B, solid arrows). (A, B) Ruthenium tetroxide; (C, D) osmium tetroxide. Scale bars: 0.5 μm.

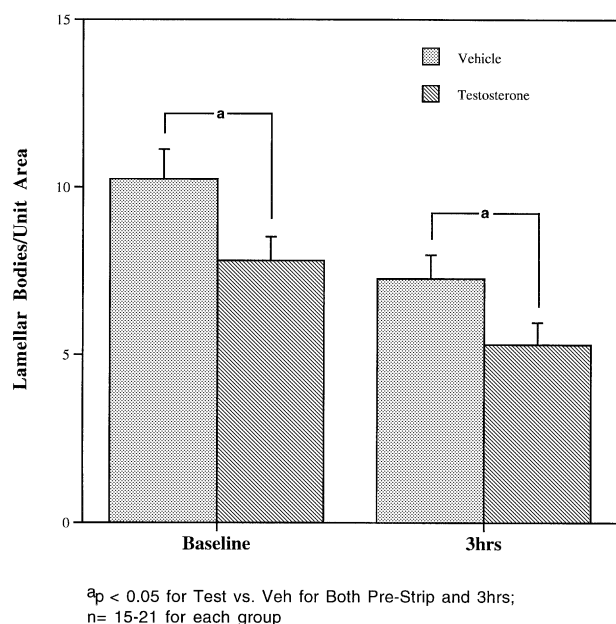


Figure 8. Lamellar body density is reduced in testosterone-replete, castrated mice. Lamellar body density in the cytosol of outer granular cells was calculated on randomly obtained, coded micrographs (n = 15–21 each). A decline in organelle density occurs after barrier disruption in both testosterone and vehicle-treated animals, but lamellar body density is significantly reduced in both testosterone-replete groups. ^ap < 0.05.

testosterone-replete, but not in vehicle-treated corneocytes (Fig 7A). Furthermore, the processing of secreted LB contents into mature lamellar bilayers appeared to be slowed (Fig 7A: note persistence of partially processed lamellar contents at level of SC 2–3 interface). Finally, as a result of decreased LB formation and secretion, the absolute quantities of extracellular lamellae in the SC interstices appeared to be reduced (Fig 7A; note decreased lamellae between SC1 and SC2). It should be noted that these changes in testosterone-replete animals also differed from normal, noncastrated male animals (data not shown), suggesting an effect of supraphysiologic levels of injected testosterone.

As in the basal state, there were no ultrastructural differences in LB formation, secretion, or postsecretory processing 3 h after acute disruption in untreated, normal *versus* vehicle-treated, castrated animals (untreated normals not shown). In contrast, there were striking reductions in LB formation, secretion, and postsecretory processing in testosterone- *versus* vehicle-treated, castrated, and control animals 3 h after barrier disruption (Fig 9C *versus* 9D; normal control not shown). Decreased LB production was evidenced by: (a) diminution in the quantities of newly secreted extracellular lamellae in testosterone-replete animals (Fig 9A *versus* 9B); and (b) the presence of lacunae in the SC interstices, which displayed decreased or absent lamellar contents (Fig 9A, asterisks). These ultrastructural observations were again validated by quantitative studies (Fig 8). Because the changes in testosterone-replete, castrated animals differed from both vehicle-treated, castrated, and untreated control skin, these findings may reflect supraphysiologic testosterone levels. Together, these results demonstrate a decrease in LB formation, resulting in additional, postsecretory alterations in testosterone-replete animals.

Finally, because acylglucosylceramide synthesis is linked to LB formation (Madison *et al*, 1998), we next assessed glucosylceramide synthesis in testosterone replete- *versus* vehicle-treated, castrated animals. As seen in Table IV, testosterone status

Table IV. Glucosylceramide synthesis in testosterone- *versus* vehicle-treated, castrated mice

| Animals | Synthesis (nmole per gm epidermal weight per h $\times 10^{-3}$) ^a | |
|-------------------------------------|---|-----------------------|
| | Total glucosylceramides | Acylglucosylceramides |
| Castrated + testosterone (n = 6) | 18.2 \pm 0.7 | 4.25 \pm 0.20 |
| Castrated + vehicle (n = 6) | 15.1 \pm 2.2 | 3.41 \pm 0.43 |
| Sham-operated (n = 6) | 20.5 \pm 2.3 | 4.62 \pm 0.55 |

^aDifferences between all groups are not significant.

modified neither bulk glucosylceramide nor acylglucosylceramide synthesis. Thus, decreased lamellar body formation with testosterone treatment cannot be ascribed to a decline in glucosylceramide production.

DISCUSSION

We show here that fluctuations in testosterone levels influence barrier homeostasis. Androgen depletion accelerated barrier recovery, and barrier function was enhanced significantly by either surgical or medical castration, indicating that these observations were independent of the method of androgen depletion. In contrast, testosterone repletion adversely influenced the kinetics of barrier recovery *versus* sham-operated controls. Androgen influenced not only barrier function, but also SC integrity, a measure of tissue cohesiveness. Although the dose of testosterone administered to hypogonadal animals generally is presumed to result in physiologic hormone levels, some of the effects that we observed could have resulted from supraphysiologic levels that occur following bolus injections. Yet, the functional differences in castrated *versus* sham-operated animals reflect changes in hormone levels that are within the normal-to-subnormal (hypogonadal) range. The physiologic relevance of these opposing outcomes in permeability barrier homeostasis was shown not only in prepubertal *versus* pubertal rodents, but also in humans.

The fact that not only systemic flutamide, but also topical antiandrogen applications accelerate barrier recovery, indicates further that the androgen effects on barrier function are directed at the skin, and not attributable to other consequences of altered circulating androgens. Epidermis expresses both androgen receptors (Mowszowicz *et al*, 1981; Coulam *et al*, 1983; Bläuer *et al*, 1991) and 5- α -reductase (Wilson and Lasnitzki, 1971; Bingham and Shaw, 1973; Dubé *et al*, 1975; Takayasu *et al*, 1980; Luu-The *et al*, 1994; Eicheler *et al*, 1995; Thiboutot *et al*, 1995), and keratinocytes can generate the more active hormone, dihydrotestosterone (DHT), if presented with testosterone (Wilson and Walker, 1969; Wilson and Lasnitzki, 1971; Sansone and Reisner, 1971). Both testosterone and DHT bind to the androgen receptor, and testosterone exerts powerful effects on skin, independent of conversion to DHT (Randall and Ebling, 1982). At present, it is not known whether the effects of testosterone on barrier function absolutely require prior intracutaneous generation of DHT. Finally, the negative effect of androgens cannot be attributed to increased sebaceous lipid secretion, since barrier homeostasis is normal in asebia mice (Mao-Qiang *et al*, unpublished observation).

Our findings in adult epidermis are completely consistent with our earlier work in fetal skin. Both *in utero* and in explant cultures, testosterone inhibits whereas estrogen enhances barrier development (Hanley *et al*, 1996). Hanley *et al* (1996) proposed further that

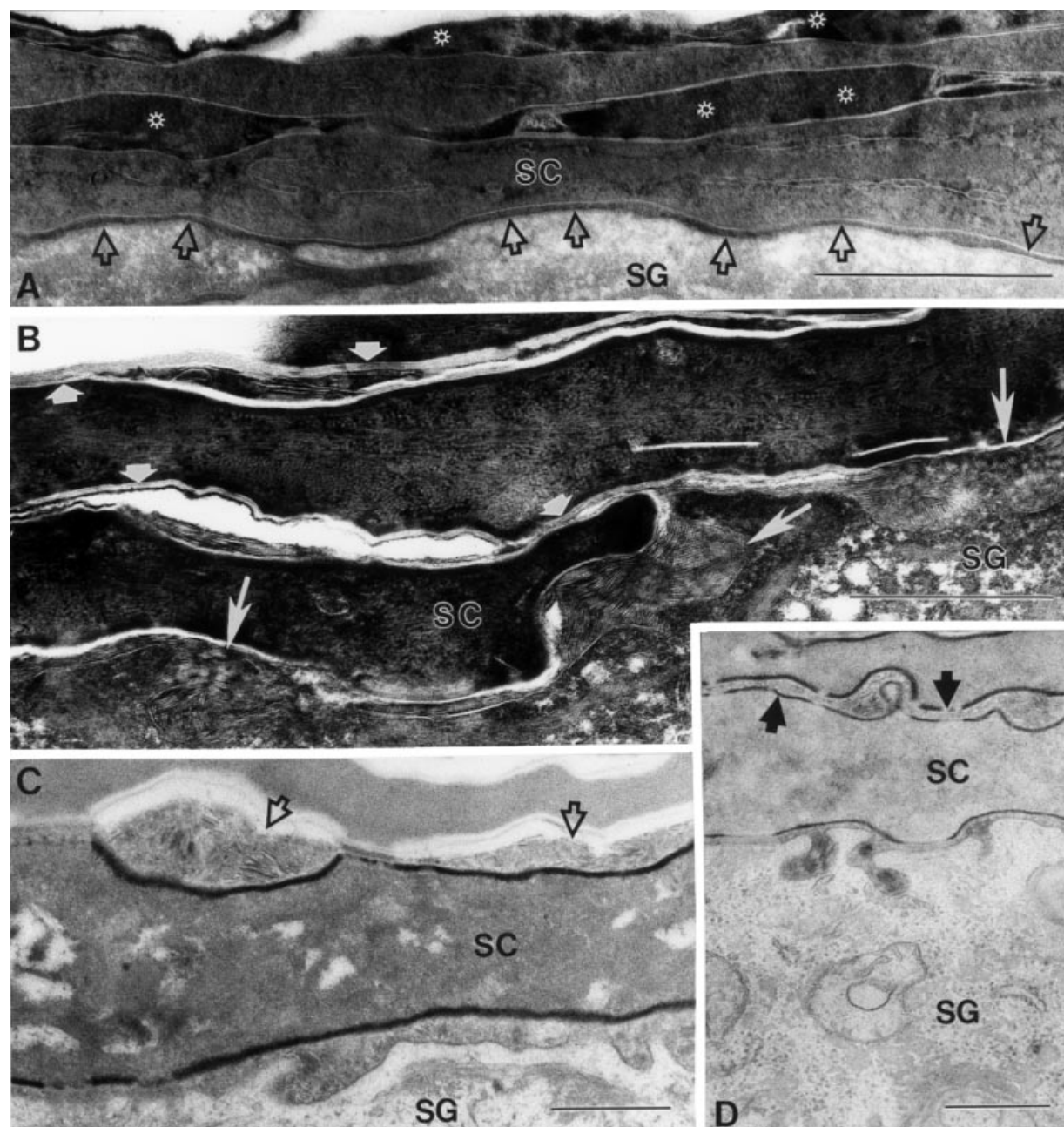


Figure 9. The lamellar body secretory response is impaired after acute barrier disruption. (A, C) Castrated plus testosterone-treated, 3 h after tape stripping; (B, D) castrated plus vehicle-treated, 3 h after tape stripping. In vehicle-treated samples, much more secreted material appears at the SG–SC interface than in testosterone-treated animals (B, solid white arrows versus A, open arrows). In addition, the extracellular processing of secreted lamellar material after acute barrier disruption appears to be delayed in testosterone- versus vehicle-treated animals (in C versus D note persistence of unprocessed material; in A versus B note replacement of mature lamellar bilayers by lacunae, A, asterisks; cf. B, small solid arrowheads = mature bilayers). (A, B) Ruthenium tetroxide; (C, D) osmium tetroxide. Scale bars: 0.5 μ m.

the opposing effects of testosterone *versus* estrogen on skin development could explain the persistence of increased mortality in premature male *versus* female infants during the surfactant-replacement era (Khoury *et al*, 1985; La Pine *et al*, 1995; Williams *et al*, 1998).

Although the consequences for the fetus seem clear, the potential clinical implications of compromised barrier homeostasis in testosterone-replete adults are still unclear. Common skin diseases, such as atopic dermatitis, psoriasis, and irritant/occupational dermatitis, appear to be as common in females as in males (Diepgen and Coenraads, 1999; Laughter *et al*, 2000). Moreover, female humans are not known to exhibit superior

barrier function to male humans (Lammintausta *et al*, 1987; Goh and Chia, 1988; Reed *et al*, 1995). Thus, there is an apparent paradox between our observations and the reported lack of gender-related, clinical, and functional differences, which could be explained by one or more of the following: (1) sufficient androgen metabolites could be generated in females to exert similar effects; or (2) estrogens could exert comparable negative effects on barrier homeostasis in adult females. Preliminary studies in female mice, however, do not support either of these possibilities: neither castration nor topical flutamide accelerate barrier recovery in adult female mice (Kao J, Elias PM, Feingold KR, unpublished preliminary observations). At least

one additional possibility remains; i.e., that gender-related differences in function could be unearthed in future studies. Indeed, one recent epidemiologic study found a male preponderance for adult psoriasis, accounted for solely by an increased disease incidence in males in the second through fourth decades (Raychaudhuri and Gross, 2000), a shift in incidence that coincides with peak androgen production in males. Moreover, gender, through its impact on barrier function, could also influence disease severity, rather than disease prevalence. Many clinical dermatologists note that severe atopic dermatitis (Paller A, Williams ML, Luck AW, personal communication, March 2000) and severe psoriasis (Krueger GR, personal communication), diseases that are associated with barrier abnormalities, occur more commonly in males than in females. Yet, the impact of gender on the severity of skin disease has not been studied systematically.

Androgens reportedly stimulate both epidermal mitogenesis and differentiation (Montagna *et al*, 1949; Eartly *et al*, 1951; Zackheim, 1968; Tammi, 1982; Tammi and Santti, 1989); however, in our system, testosterone repletion instead induced a modest decrease in epidermal thickness. Whether this observation contributes to the testosterone-induced delay in barrier recovery is unclear. Certainly, a decrease in the number of nucleated cell layers could result in fewer cells available for lamellar body production (see below). Our functional observations cannot, however, be explained by altered epidermal or sebaceous lipid synthesis, because: (a) epidermal lipogenesis was comparable in testosterone-replete *versus* hypogonadal animals, these results are consistent with prior observations that lipid synthesis is actually more robust in male *versus* female mammalian epidermis (Feingold *et al*, 1982, 1983); (b) topical flutamide actually inhibits lipogenesis in sebaceous glands (Lutsky *et al*, 1975); and (c) barrier homeostasis is normal in asebja-J1 mice, which demonstrate profound sebaceous gland hyperplasia.²

In the absence of a change in lipid synthesis abnormality, we examined alternate, distal mechanisms as contributors to the androgen effects on barrier homeostasis. In addition to lipid synthesis, barrier homeostasis requires the assembly and secretion of epidermal lamellar bodies, followed by postsecretory, extracellular processing (Elias and Menon, 1991; Elias and Feingold, 1992; Elias, 1996). The epidermal lamellar body is a unique secretory organelle that delivers lipid precursors and hydrolytic enzymes to the SC interstices, leading to barrier formation (Elias and Menon, 1991; Elias and Feingold, 1992). This organelle is a product of an extensive trans-Golgi network in the apical cytosol of the outermost SG (Elias *et al*, 1998), which amplifies organelle secretion in response to barrier perturbation (Elias and Feingold, 1992; Elias *et al*, 1998). In addition to a potential reduction in the number of cells available for LB formation, we found a significant reduction in LB density/cell in testosterone-treated animals; however, despite the acknowledged importance of acylglycosylceramide production for LB generation (Madison *et al*, 1998), synthesis of glucosylceramides did not decline in parallel with reduced LB formation in testosterone-replete animals.

In addition to decreased LB production, we observed a downstream diminution in LB secretion and postsecretory, extracellular processing. The observed decrease in secretion could simply reflect decreased LB production, or alternatively the reduction in LB secretion could result from other mechanisms; e.g., testosterone-induced changes in the epidermal calcium gradient (Menon *et al*, 1985), which directly regulates

LB secretion (Menon *et al*, 1994). Further mechanistic studies will be required to delineate the apparent link between changes in testosterone levels, barrier function, and LB production, as well as the basis for the observed androgen effects on SC integrity (cohesion).

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